Grape berry cv. Shiraz Epicuticular Wax and Transpiration during Ripening and Preharvest Weight Loss

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Abstract: Preharvest weight loss of Vitis vinifera L. cv. Shiraz berries may be the result of cuticle disruption leading to high transpiration rates relative to earlier stages of ripening. Scanning electron microscopy showed very few but functional stomata on young berries and wax-filled stomata on older berries and, aside from slight cracks along the stomatal protuberance, did not reveal any fissures in the surface of berries that may lead to increased transpiration rates. Preveraison berry epicuticular wax platelets were defined and intricate, while postveraison and shriveled berry surfaces had large areas of amorphous waxes. Postveraison, the area of amorphous wax relative to intricate wax was not correlated with berry age or degree of shrivel. Extraction of surface waxes revealed that total wax on a surface-area basis decreased during veraison then remained stable as the berries ripened and entered the weight-loss phase. Berry transpiration, estimated from loss of fresh weight of detached berries over time, during this final shriveled phase was 16% of the preveraison rate on a per berry basis. Nevertheless, berry transpiration could account for an average 15 mg loss in fresh weight per berry per day. We conclude that weight loss during late ripening of Shiraz berries was not the result of cuticle disruption or high transpiration rates alone. It is hypothesized that decreased vascular flow of water into the berry combined with continued transpiration leads to the weight loss.

Key words: Vitis vinifera, berry, shrivel, transpiration

Grape berries can lose weight and shrivel at advanced stages of maturity. Berries of Vitis vinifera L. cv. Shiraz are unusual in that the weight loss can occur well before the berries are suitable for harvesting, even under adequate irrigation (McCarthy 1999). This weight loss can begin 80 to 115 days after flowering, depending on season, and can reach up to 30% of maximum weight prior to harvesting. Nearly all of the weight loss (90%) is in the form of water vapor, while loss in dry weight could be accounted for by berry respiration (Rogiers et al. 2000). Impeded vascular flow from the vine into the berry during the later growth stage has been hypothesized to play a role in shriveled at suboptimal Brix (McCarthy and Coombe 1999). Unusually high berry transpiration rates may be another physiological cause for this condition, leading to excessive water loss (During et al. 1987, Lang and Thorpe 1989). If influx of water to the berry through the pedicel cannot compensate for water loss through the cuticle, then the shriveling symptoms often observed in Shiraz may result.

The cuticular membrane covers all aboveground parts of terrestrial plants, including their fruits. This membrane consists of a polymer matrix (cutin), polysaccharides, and solvent-soluble lipids (cuticular waxes) (Holloway 1982). The cuticle controls water movement between the epidermal cells and the ambient atmosphere (Riederer and Schreiber 2001). When present, stomata are the preferred sites of water loss. In grape berries, however, their frequency is even lower than that of leaves of crassulacean acid metabolism plants (Blanke and Leyhe 1987), and it is likely that the cuticle is important in controlling water loss. The structural arrangement of the wax, together with its chemistry, controls the water movement from berries (Chambers and Possingham 1963, Possingham et al. 1967). The structure of epicuticular waxes changes with fruit age. Although normally crystalline, the wax material is rather soft and can be altered or removed by the impact of rain, abrasion from wind-blown particles, or contact with other berries, leaves, or other adjacent objects. On some leaf surfaces investigated, the crystals have appeared to degrade slowly over time, fusing into amorphous masses and eventually into a continuous layer on top of the normal, uninterrupted wax layer (Reicosky and Hanover 1976, Grill et al. 1987).

Reynhardt and Riederer (1991) proposed a model of the physical structure of the epicuticular wax layer, describing it as a matrix composed of highly ordered crystalline and disordered amorphous regions. Reduced diffusional dynamics in the crystalline regions of the wax barrier make them practically impermeable. Thus, transport across the wax barrier may be expected to occur only through the amorphous regions. The physical state of the wax layer, more than its chemical composition, may determine the transport properties of the
cuticle. It is therefore important to investigate the relative
distribution of crystalline and amorphous regions on the
Shiraz berry and to determine if a potential transition from
crystalline to amorphous wax is related to the shrivel phe-
omenon. Moreover, cuticle disruption in fruits can be due
to microbial pathogens or sun exposure and extreme tem-
peratures. Cracks in the cuticle of expanding berries have
also been reported for some varieties (Percival et al. 1993,
Comménil et al. 1997). The presence of these cracks would
provide open wounds, and once damaged, the berry may be
more susceptible to high rates of water loss.

The objectives of the present work were to examine the
surface of the Shiraz berry during late ripening and shrivel-
ing for evidence of cuticular disruption and to correlate
properties of the waxy cuticle with transpiration of indi-
vidual berries during development.

Materials and Methods

Berry weight. Five bunches of berries were harvested at
random from field-grown Shiraz vines twice weekly from
flowering to harvest over the 1999 to 2000 and the 2001 to
2002 seasons. These 11-year-old, own-rooted vines were
from clone PT23/N/Griffith and were located at Charles Sturt
University, Wagga Wagga, NSW, Australia. Fifty-berry sam-
ples were taken at random from each bunch and weighed.

Epicuticular wax extraction. Fifty berries from three
bunches of each sampling date of the 1999 to 2000 season
were weighed and briefly rinsed in water to remove dirt and
dust. They were dried on filter paper and then dipped in
chloroform three times in separate vials (30 sec, 10 sec, 1
sec). Chloroform extracts were combined in tarred vials and
allowed to evaporate at 22°C under nitrogen flux overnight
and then dried at 40°C for 5 to 7 days until constant weight.
Water-soluble exudates were partitioned from the waxes by
dissolving in water (5 mL), then chloroform (5 mL). The wa-
ter layer was removed with a pipette and the chloroform
layer was dried as described above.

Scanning electron microscopy. Shiraz berries were
sampled during the 1999 to 2000 season at random from the
field vines described above, taking care not to make contact
with the surface of the berry. Vitis vinifera L. cvs. Cabernet
Sauvignon and Chardonnay berries were also sampled from
the same vineyard. Some berries were plunged into liquid
nitrogen, and 1-cm² skin sections were freeze-dried, at-
tached to aluminum stubs, and sputter-coated with gold at
0.05 mbar and 25 mA for 3 min prior to scanning electron
microscopy (SEM). In a second method, a 1-cm² slice was
cut from each berry, attached to a flat brass stub, quick-fro-
zened at approximately -170°C on the cold stage of a JEOL
6400 SEM (JEOL Australasia Pty Ltd, Sydney, Australia),
gold-coated, and then observed.

Transpiration. Berries of five developmental stages (six
replicates) from preveraison to shriveled were harvested at
random from the field vine described above during the
2001 to 2002 season. Height and width of each berry were
measured with callipers, and surface area was estimated
according to the modified equation for a sphere: surface
area = π(width)(height). Pedicels were dipped in paraffin
wax to prevent water loss from this exposed surface. Berries
were suspended in an incubator at 25°C with silica gel to
maintain a constant 23% relative humidity without air turbu-
lence. Berries were weighed twice daily over three days, and
transpiration rates were calculated from the slope of the lin-
er regression of weight loss over time (the r² of each slope
was ≥0.994 ), taking into account an average daily respira-
 tion rate of 1.9 mg CO₂ per berry (Niimi and Torikata 1979).

Results

Berry weight. In the 1999 to 2000 season, gain in berry
fresh weight was biphasal with a central transition point
(Staudt et al. 1986, Blanke 1992) one week before veraison
(67 days after flowering, DAF) (Figure 1A). Mean berry
fresh weight reached a maximum at 95 DAF (1.5 g), and then
decreased by 20% to 1.2 g at 115 DAF, giving an average
weight loss of 15 mg per berry per day. In the 2001 to 2002

Figure 1 (A) Berry fresh weight (n = 5, 50 berries per sample) and (B)
berry epicuticular wax amounts (n = 3, 50 berries per sample) during
development and ripening. Inset refers to mass of chloroform extract
prior to partitioning with water to remove berry exudates (n = 3, 50
berries per sample). Bars indicate standard error of the mean.
season, veraison again occurred at 67 DAF and the berry weight maximum occurred at 106 DAF (1.6 g). Berries were harvested at 133 DAF when they weighed 1.2 g. Weight loss was 25%, with an average weight loss of 15 mg per berry per day as in the 1999 to 2000 season.

**Epicuticular wax.** Mass of the primary chloroform extract per unit berry surface area prior to water extraction was 1.4 ± 0.1 µg mm⁻² and did not change through development (Figure 1B). After removal of water-soluble berry-surface exudates from the chloroform extract, mass on a surface-area basis ranged between 1.1 and 1.2 µg mm⁻² from 35 to 50 DAF, then declined by 90 DAF (berry weight maximum) to 17% of peak values (0.2 to 0.4 µg wax mm⁻²) (Figure 1B) and remained low through the weight-loss phase until berries were harvested at 110 DAF. In contrast, the water-soluble exudates increased from approximately 18% of the total surface exudates at 35 to 50 DAF to 83% of exudates by 90 DAF.

**Morphology of the grape berry.** The morphology of the grape berry changed during fruit ontogeny and ripening. At anthesis the ovary was covered in cuticular ridges (Figure 2A), which had spread out by 20 DAF (Figure 2B) and wax was present as grains (Figure 2C). Occasional protuberant stomates (on average less than five per berry) were also visible (Figure 2D). Stomates were visible on the flower cap prior to bloom, on the ovary, and on the preveraison berry. These stomates were hidden once the epicuticular waxes covered the berry. One or two stomates could be seen on postveraison berries, but they were mostly filled by the wax (data not shown). At 40 DAF the waxes formed well-defined plates with fringed edges and were so dense that the cuticle membrane underneath was no longer visible (Figure 2E, F, G). In the postveraison berry, the cuticle and epicuticular wax was approximately 2-µm thick (Figure 2H). The defined vertical platelets also featured in the postveraison and post-weight maximum berries (Figure 3A, B, C) and were similar to those seen on Cabernet Sauvignon (Figure 3D) and Chardonnay (data not shown). From veraison onward, large areas of amorphous wax were also observed in all three varieties (Figure 3E). The top ends of the vertical platelets appeared to spread into umbrella-like horizontal plates that condensed to form a smooth crust (Figure 3F, G). These smooth areas covered from 10 to 80% of the berry and the area was not dependent on the age of the postveraison berry or the degree of shrivel. However, sun-exposed berries had larger areas of the amorphous wax than shaded berries. The wax was also amorphous in those areas where berries were in contact with other berries.

Cavities or fissures were not visible in plump, slightly shriveled, or moderately shriveled Shiraz berries. However, there were tiny cracks along some of the stomatal protuberances in several of the postveraison berries. Moreover, very tightly shriveled berries also had cavities in the surface (Figure 3H).

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**Figure 2** Scanning electron micrographs of grape berry surfaces at various developmental stages. (A) Cuticular ridges on the surface of Shiraz ovary at anthesis. (B) Surface of Shiraz preveraison berry at 20 DAF. (C) Close-up of (B) showing cuticular ridges and rudimentary wax granules. (D) Stomate on berry surface at 20 DAF. Note the lack of wax granules near the opening. (E–H) Typical berry surface at 40 to 120 DAF featuring upright, intricate scalelike wax platelets. (H) Transverse section of the dermal and cuticle layers of a postveraison berry; cuticle, including epicuticular wax layer, ~2 µm thick. Bar in A, F, H = 10 µm; bar in B, D = 20 µm.; bar in C, G = 5 µm; bar in E = 100 µm.
Transpiration. From the preveraison to the post-veraison phases of berry development, transpiration rates per unit surface area decreased to 19% of original rates (Table 1), which then decreased by another 3% during the shriveled phase. On a per berry basis, transpiration of shriveled berries was 16% of preveraison rates and was equivalent to 15.2 mg berry⁻¹ day⁻¹.

Discussion

The epicuticular wax development on Shiraz berries in this study was similar to that described earlier for other varieties, for example, cvs. Thompson Seedless (Rosenquist and Morrison 1988) and Palomino fino (Casado and Heredia 2001). Cuticular ridges were present at flowering, and these spread out and fused as the berry expanded. Rosenquist and Morrison (1988) suggested that the cuticular ridges function as a storage form of cuticular material, which spreads as the berry expands. The first epicuticular wax appeared as small grains and then formed into intricate crystalline platelets 20 to 40 days after flowering. This was followed by the development of an amorphous wax layer on top of the existing platelets post-veraison. The proportion of the berry surface that was covered with amorphous wax was larger on sun-exposed berries than on shaded berries, perhaps related to the substantially higher temperature experienced by the exposed berries. Such berries often reach 15°C above ambient temperature, whereas shaded berries are generally close to ambient temperature (Smart and Sinclair 1976, Spayd et al. 2002). The surface wax of Valencia orange fruit changed from upright plates to amorphous during development, a change that occurred earlier for exposed fruit (El Otmani et al. 1989). There were extensive areas of amorphous wax on postveraison berries in our study. However, shriveled berries did not have more extensive amorphous wax than plump postveraison berries, suggesting that shriveling in Shiraz berries is not the result of amorphous cuticular wax development.

Fissures on skins of berries may lead to increased transpiration rates. Fissures were observed in cvs. Optima (Percival et al. 1993) and Pinot noir (Comménil et al. 1997) skins at maturity. Our investigation revealed no fissures along the surfaces of postveraison, mildly shriveled, or moderately shriveled Shiraz berries. Cabernet franc berries are relatively small, and like Shiraz berries in this study, these did not show any fissures traversing the cuticle (Percival et al. 1993). Cuticles of varieties with larger berries may be more susceptible to fissures because there is lack of adequate mechanical support (Percival et al. 1993). In some varieties small cracks can develop at the base of stomatal protuberances during veraison (Blanke et al. 1999). We also observed these cracks on some post-veraison berry stomata, but, as this phenomenon occurs in many varieties, it is unlikely that it would lead to the shriveling that is unique to Shiraz. The cavities in the se-
Table 1  Transpiration rates of five developmental stages of Shiraz berries sampled on the same day. Loss in fresh weight of detached berries monitored over three days at 25°C and 23% relative humidity (VPD = 2.45 x 10⁻³ MPa). Transpiration rate calculated by subtracting a daily respiratory loss of 1.9 mg CO₂/berry (Niimi and Torikata 1979) as carbon from fresh weight loss. Values are means ± standard errors.

<table>
<thead>
<tr>
<th>Berry maturity</th>
<th>Soluble solids* (Brix)</th>
<th>Transpiration rate (µmol m⁻² s⁻¹)</th>
<th>Transpiration rate (µmol berry⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green (preveraison)</td>
<td>b 129 ± 3</td>
<td>0.062 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>Pink (50%)</td>
<td>22.6 ± 0.7</td>
<td>62 ± 2</td>
<td>0.030 ± 0.001</td>
</tr>
<tr>
<td>Pink (100%)</td>
<td>22.6 ± 0.7</td>
<td>47 ± 2</td>
<td>0.023 ± 0.001</td>
</tr>
<tr>
<td>Blue (postveraison)</td>
<td>31.2 ± 0.9</td>
<td>24 ± 1</td>
<td>0.0117 ± 0.0006</td>
</tr>
<tr>
<td>Shriveled</td>
<td>44.0 ± 0.8</td>
<td>21 ± 1</td>
<td>0.0098 ± 0.0006</td>
</tr>
</tbody>
</table>

*aSoluble solids readings taken after three days of transpiration measurements.

*bPreveraison berries did not contain adequate amounts of juice for a soluble solids assessment.

As indicated above, in other varieties there appears to be no consistent correlation between berry development and wax thickness or quantity per surface area. For example, the wax of Delaware grapes increased during the early preveraison stage (until 30 DAF) and then remained constant thereafter (Yamamura and Naito 1983). In Thompson Seedless grapes, however, no change in the total wax (1 µg mm⁻²) during berry growth was reported (Radler 1965), and an increase in the weight of wax per unit surface area occurred during development of Pinot Noir grapes (Comménél et al. 1997). Our results for Shiraz suggest that the formation and deposition of epicuticular wax occurs early during berry development and ceases before the central transition point of berry growth. These conflicting reports on amount of berry surface wax may be due to differences in methods of wax extraction. Furthermore, the length of the developmental period investigated and environmental factors such as temperature, light exposure, relative humidity, and irrigation (Rosenquist and Morrison 1989) will have an effect on trends.

One might intuitively assume that the amount of epicuticular wax will affect cuticular transpiration. In this study, however, there was no negative correlation between amount of wax and berry transpiration. Berry transpiration per surface area decreased from preveraison to postveraison and then decreased even further during the shriveling phase of ripening. Similarly, transpiration of postveraison Müller-Thurgau (Leyhe and Blanke, 1989) and Cabernet Sauvignon (Greenspan et al. 1994, 1996) berries was less than that of preveraison berries. The composition of the cuticle and the epicuticular wax may be more important than its thickness or density for determining transpiration rates. The physical structure of the wax, such as crystallinity, may also affect transpiration rates (Reynhardt and Riederer 1991), but as there was no correlation between area of amorphous wax and incidence of shrivel, other factors may be involved.

Loss of stomatal functioning may also contribute to the decreased transpiration during ripening of grape berries (Palliotti and Cartechini 2001). Shortly after fruit set, the stomata were raised on a conical protuberance and appeared to be functional. An “aureole peristomate” was first described for the grape berry by Bessis (1972), and its protuberance was discovered and labeled “stomatal protuberance” by Blanke and Leyhe (1988). The very few stomata we observed in postveraison berries in this study had been transformed into nonfunctional lenticels and were primarily filled with wax. The continued decrease in berry transpiration after veraison is further evidence that shrivel is not caused by fissures or cavities in the skin. Such physical damage would be expected to increase transpirational water loss rather than to decrease it.
Transpiration rates of shriveled berries were equal to the rates of weight loss observed in field berries (on average 15 mg water vapor berry⁻¹ day⁻¹). However, since transpiration rate decreased on both a surface-area and whole-berry basis during ripening, it is unlikely that this alone would be the cause for shrivel. In a growing postveraison berry (prior to the weight maximum), net water flow into the berry must exceed net water loss. In a shriveling berry, the rate of water loss through transpiration was slightly less than that of the growing postveraison berry, indicating that net water flow into this shriveling berry must have been less than that of the growing postveraison berry. If transpiration rates had suddenly increased during shriveling, then transpiration alone could have accounted for the transformation of a plump berry into a shriveled one. While Coombe and McCarthy (2000) have hypothesized that xylem flow into the berry decreases at veraison and that phloem flow is discontinued at the weight maximum, earlier studies using element accumulation patterns indicated that xylem flow into the berry is not necessarily completely impeded after veraison (Ollat and Gaudillère 1996, Rogiers et al. 2000). Tracer dye studies pointed to a xylem disruption between the brush region of the berry and the rest of the berry (Rogiers et al. 2001). However, this disruption does not necessarily indicate a complete cessation of xylem exchange between the vine and the berry since water could potentially flow into the berry through the xylem up to the brush and then continue along symplastic routes (between adjacent cells through plasmodesmata) from the brush into the pulp and skin regions. Although not thought to be important after veraison (Greenspan et al. 1994), backflow has been reported in grapes (Lang and Thorpe 1989) and may play a role in shrivel. Further elucidation of this phenomenon, together with data on rates of phloem flow into the berry, is required to better understand the physiological cause of shrivel in Shiraz berries.

Conclusion

The objective of this study was to examine the role of berry transpiration in Shiraz berry shrivel. Epicuticular wax amounts on a surface area basis declined after veraison, but did not decline further after the berry weight maximum. The epicuticular wax platelets were intricate or amorphous, with no correlation between the area of amorphous wax and incidence of shrivel. There were no fissures in the surface of the cuticle that might lead to increased transpiration rates. Small cracks were visible along the stomatal protuberance, but that was not likely associated with shrivel as the cracks were present in preweight maximum berries and are fairly common among other grape varieties. As fruits ripened and subsequently shriveled, there was a decline in transpiration on both a whole-berry and surface-area basis. Shrivelning in Shiraz was not caused by a sudden spike in transpiration rates. Rather, we hypothesize that a decrease in net vascular flow into the berry together with continued transpiration leads to the weight loss.

Literature Cited


